THE METABOLISM OF LUNG TISSUE IN EXPERIMENTAL LOBAR PNEUMOCOCCUS PNEUMONIA IN THE DOG*

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One of the outstanding features of pneumococcus lobar pneumonia is the intensity and magnitude of the inflammatory reaction which persists for a week or more and which in most instances subsides without any apparent damage to the tissue. The inflammation profoundly alters the conditions under which the tissue must metabolize. The alveoli are filled with exudate which is composed principally of leucocytes and which usually contains large numbers of pneumococci. The change in the alveoli is from a completely aerobic to an almost completely anaerobic environment. Oxygen for tissue respiration, which formerly was obtained from alveolar air, now must be derived mainly from venous blood. The leucocytes and pneumococci (Friedemann, 1) metabolize at a very rapid rate. The blood which formerly provided nutrients for the alveolar cells must now provide adequately for a greatly increased number of rapidly metabolizing cells; also, if the tissue is to survive, metabolites and toxic substances must be removed.

To what extent do the severe conditions imposed on the lung affect the metabolism of the tissue? Does the blood adequately supply oxygen and the necessary nutrients; and are the metabolites efficiently removed? To answer these questions we have analyzed, first, the tissue at various stages in the disease, and secondly, the blood entering and leaving the consolidated as well as the uninfected lung.

EXPERIMENTAL

Experimental Pneumococcus Pneumonia.—This was produced in dogs by the method of Terrell, Robertson, and Coggeshall (2). A highly virulent Type I

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organism (A_5) was used throughout. It was found impossible to produce constantly lesions confined to single lobes, due to the natural variability in the experimental disease. Therefore, in order to classify the lesions according to their respective stage of development x-ray exposures were made at least once a day, and, at death, representative sections were studied histologically.

Analysis of Tissues.—The animals were killed by a 220 volt electric current applied for a period of 7 seconds. The electrodes, which consisted of heavy clamps, were attached to the lip and hind leg after moistening with salt solution. The chest was then immediately opened. Blood was withdrawn from the heart and representative samples of lung tissue were simultaneously dropped into Dewar flasks which contained liquid nitrogen. With sufficient assistance we were able to carry out the sampling in a minimum of 45 seconds, with an average for all experiments of about 90 seconds, from the time of application of the current. Immediately following this the exudate was vitally stained by the technique of Kredel and Van Sant (3). Specimens were then taken for histological examination.

The frozen samples were crushed and prepared for analysis by the technique of Graeser, Ginsberg, and Friedemann (4). The following analytical methods were used: lactic acid, Friedemann and Graeser (5); glucose, Shaffer-Hartmann as modified by Shaffer and Somogyi (6); glycogen, Somogyi (7).

As a control on the technique, blood was taken from the saphenous vein of 6 of the animals before electrocution, and from the heart after death. The glucose, lactic acid, and non-protein nitrogen content of both samples agreed in all cases within the limits of experimental error of the methods. It would seem that the electric current arrests cardiac motion almost instantly, thus preventing further circulation of blood. The necessity for simultaneous analysis of blood and tissue has been pointed out by Graeser, Ginsberg, and Friedemann. It is a valuable aid in the comparison of data from various animals and provides a measure of postmortem changes within the tissues.

Analysis of Blood from Pulmonary Vein.—The left lower lobe was infected in the usual manner. Surgical anesthesia was induced by nembutal. The sternal plate was removed, exposing the heart and lungs. Artificial respiration was immediately begun. Although the number of induced respirations was about 20 per minute the lungs were not fully inflated. While it may have resulted in less complete oxygenation of the blood, this procedure was nevertheless followed for reasons which will be given in the discussion. Blood was withdrawn from the right ventricle, from the pulmonary vein of an uninfected lobe, and from the pulmonary vein of the infected left lower lobe. The blood vessels of this lobe are more accessible and less manipulation is required to obtain the sample. Cold syringes were used, previously oiled and freed of air and fitted with long No. 18 gauge needles. Needles, bent at right angle about 1.5 inches from the tip, were used to obtain the pulmonary blood. A temporary ligature, a loop around the pulmonary vein, may be applied during collection of the sample. The blood was immediately emptied into cold tubes containing oil and the necessary quantities of sodium oxalate and sodium fluoride.

RESULTS

Analysis of Tissues.—Normal lung tissue, perhaps more than any other tissue, carries on its functions in an almost completely aerobic environment. This is especially true of the cells which line the alveoli. The aerobic character of its metabolism is indicated by the following data recalculated from Table I and shown in Table II. Excluding the data from dog 1, the averages from 10 animals expressed as mg. per cent, for the normal lung tissue were glucose 72,[1] lactic acid 15; the averages for blood were glucose 84, lactic acid 15. Assuming that one-fifth of the normal tissue analyzed was blood,[2] the calculated concentrations of glucose and lactic acid within the tissue itself were 69 and 15 mg. per cent respectively. In spite of the great vascularity of the tissue and the rapid diffusion of glucose from the blood into the tissue, the normal tissue contained an average of 14 mg. per cent less of glucose. This indicates a rapid metabolism of glucose. Lactic acid either was not produced at all, or else its rate of production was balanced by its rate of oxidation. Altogether, the results indicate a completely aerobic metabolism of carbohydrate.

Entirely different results were obtained from the consolidated tissue. This was to be expected for the growth of the bacterium brings about a profound change in the environment. Although the number of pneumococci at first may be relatively small, there is an immediate response resulting in engorgement of the capillaries and filling of the alveoli with serous exudate (9, 10). Whether the initial edema represents an anaphylactic reaction or whether it is due to an edema-producing irritant (11) cannot be determined with the data at hand. The growth of the pneumococcus appears to be very rapid at this stage, for many organisms can be seen in the exudate. Almost simultaneously great numbers of polymorphonuclear leucocytes enter the alveoli until the latter become packed with leucocytes; the organisms are at the same time rapidly removed by phagocytosis. With

[1] That the reducing power of zinc filtrates from tissues is due largely to glucose was shown recently by Blatherwick (8). The average values for the non-fermentable reducing substances, expressed as mg. per cent of glucose, were as follows: liver, 11.7; muscle, 12.0; kidney, 7.6; blood, 4.9.

[2] Although the correctness of this assumption, in the absence of hemoglobin determinations, may be questioned, the results are not greatly affected by calculations of this kind, nor are the conclusions materially altered.

TABLE I

Experimental Pneumococcus Lobar Pneumonia in Dogs

Dog	Time after onset of infection	Tissue	Analysis of tissues			Histological appearance of infected tissue*	Fresh exudate cells		
			Glucose	Lactic acid	Glycogen		Polymorphonuclear	Mononuclear†	Living cells
	days		*mg. per cent*	*mg. per cent*	*mg. per cent*		*per cent*	*per cent*	*per cent*
1	2	Infected	28	41		Uniform exudate of pmn. cells			
		Normal	48	41					
		Blood	49	21					
2	2	Infected	41	50		Uneven exudate, predominantly pmn. cells	85	15	74
		Normal	81	21					
		Blood	100	19					
3	3	Infected	46	41	547	Uniform exudate of pmn. cells	86	14	92
		Normal	67	11	136				
		Blood	69	10					
4	3	Infected	80	24		Uniform exudate, predominantly pmn. cells			75
		Normal	81	11					
		Blood	97	11					
5	4	Infected	62	43	227	Uneven exudate, predominantly pmn. cells	90	10	77
		Normal	72	26	186				
		Blood	83	27					
6	4	Infected	63	25	374	Uniform exudate of pmn. cells	79	21	96
		Normal	74	11	145				
		Blood	88	10					
7	4	Infected	52	19		Advanced resolution. Marked macrophage reaction	33	67	94
		Normal	63	14					
		Blood	81	11					
8	5	Infected	58	18		Advanced resolution. Exudate of mononuclear cells	33	67	52
		Normal	58	8					
		Blood	76	11					

* In this column, pmn. refers to polymorphonuclear cells.

† This includes lymphocytes, monocytes, and macrophages.

TABLE I—*Concluded*

Dog	Time after onset of infection	Tissue	Analysis of tissues			Histological appearance of infected tissue*	Fresh exudate cells		
			Glucose	Lactic acid	Glycogen		Polymorphonuclear	Mononuclear†	Living cells
	days		*mg. per cent*	*mg. per cent*	*mg. per cent*		*per cent*	*per cent*	*per cent*
9	6	Infected	60	14		Advanced resolution. Scanty exudate of mononuclear cells	17	83	71
		Normal	72	11					
		Blood	87	12					
10	6	Infected	62	21		" "	13	87	89
		Normal	72	20					
		Blood	72	17					
11	7	Infected	59	27		Advanced resolution. Exudate principally of macrophages	15	84	76
		Normal	80	16					
		Blood	84	17					
12	7	Infected			181	Beginning resolution. Exudate contains pmn. cells and macrophages			
		Normal			123				
		Blood							
13	13	Infected	90	12	133	Normal			
		Normal	97	10	128				
		Blood	102	10					
14		Normal			119	Normal			

the filling of the alveoli by exudate, the metabolism is aerobic only in so far as oxygen can be obtained from the blood supply. The situation is further complicated by the fact that pulmonary blood for the most part represents venous blood, and therefore contains less oxygen. If now the circulation should be diminished (12), as is usually considered to be the case in edematous tissues, the available carbohydrate (free sugar and perhaps also glycogen) is rapidly used up by the leucocytes, pneumococci, and, to a lesser extent, the tissue cells. Toxic products and acids thus accumulate. Should the stasis be too great, or should occlusion of blood vessels occur, the carbohydrate would soon be exhausted and the result would be death and necrosis

of the tissue. The pneumococcus can continue to grow at an apparently undiminished rate even after all of the free sugar is metabolized (Friedemann and Sutliff, 13); its source of energy probably is the protein-sugar, a polysaccharide (14), of which from 250 to 1000 mg. per cent are present in serum.

The extent of the metabolism, and its departure from the normal, in the initial stage of consolidation is strikingly shown by the data from dogs 2 to 6. See Table I. The reducing sugar (glucose) was in every case lower in the infected than in the normal tissue; the lactic acid

TABLE II

Recalculated Data from Table I

Stage of disease	Animals	Tissue	Averages		Recalculated data*			
			Glucose	Lactic acid	Glucose	Lactic acid	Difference over blood	
							Glucose	Lactic acid
			(1)	(2)	(3)	(4)	(5)	(6)
			mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
Before resolution	2 to 6	Blood	87	16	87	16		
		Normal lung	75	16	72	16	15	0
		Infected lung	58	37	55	39	32	23
During resolution	7 to 11	Blood	80	14	80	14		
		Normal lung	69	14	66	14	14	0
		Infected lung	58	20	55	21	25	7

* These are based upon the assumption that the normal tissue contains 20 per cent and the infected tissue 10 per cent of blood.

content was greatly increased. The averages of all determinations for glucose and lactic acid are shown in Table II. Assuming that one-tenth[2] of the consolidated tissue was blood, the average content of glucose and lactic acid, exclusive of blood was 55 and 39 mg. per cent respectively. Compared with blood, the infected tissue contained an average of 32 mg. less of glucose and 23 mg. more of lactic acid than the blood. About 70 per cent of the glucose was thus converted anaerobically into lactic acid. On the other hand, the normal tissue in the same groups of dogs contained 15 mg. per cent less of glucose and no increase whatever of lactic acid. The increased sugar con-

sumption was no doubt due to the great number of leucocytes and pneumococci in the exudate. The polymorphonuclear leucocytes metabolize glucose at an extremely rapid rate and, according to Barron and Harrop (15), produce large quantities of lactic acid even in a completely aerobic environment. Yet in spite of the greatly increased metabolism the tissues still contained carbohydrate and a relatively slight increase in the concentration of lactic acid. It is apparent that the circulation of blood is sufficient to allow ample carbohydrate to diffuse into the tissue and, by diffusion outward, to remove the metabolic products.

The recovery was accompanied by a marked macrophage reaction, in confirmation of the studies by Robertson (9, 10, 16) and Kredel and Van Sant (3). From Table I it can be seen that the mononuclear cells (mostly macrophages), which at first constituted only about 10 per cent of the exudate cells, increased in numbers until at the time of resolution they constituted more than two-thirds of all cells. At the time of analysis the tissues still contained some exudate and many cells, as was shown by x-ray and by microscopic examination of sections. The condition of the animals was greatly improved. The individual data in Table I still show a higher metabolism of carbohydrate in the infected than in the normal tissue. This was to be expected since the exudate still contained many cells. On the other hand, the lactic acid content was reduced. This is best shown by the recalculated data from dogs 7 to 11 in Table II. The average difference between the reducing sugar of blood and tissue is 25 mg. per cent; the difference in lactic acid content is only 7 mg. per cent. This, we believe, points to an almost complete reestablishment of aerobic conditions. A certain amount of lactic acid is still to be expected, since the diffusion of oxygen from the blood into the exudate contained in the alveoli is not rapid enough to prevent entirely lactic acid formation. But even if the environment were completely aerobic a certain amount of lactic acid would still be formed, for Barron and Harrop (15) have shown that macrophages produce some lactic acid from sugar in an aerobic environment.

Analysis of Blood from Pulmonary Vein.—Results from two animals are shown in Chart 1 and Tables III and IV.

It was realized in these experiments that the conditions could not be the same as in the intact animal. They were decidedly abnormal due to the deep nembutal anesthesia, a rapid loss of blood (some of which was unavoidable because of the operative procedure), and to the under-ventilation. The lungs were under-ventilated (*a*) to avoid unnecessary manipulation of the heart and lungs during

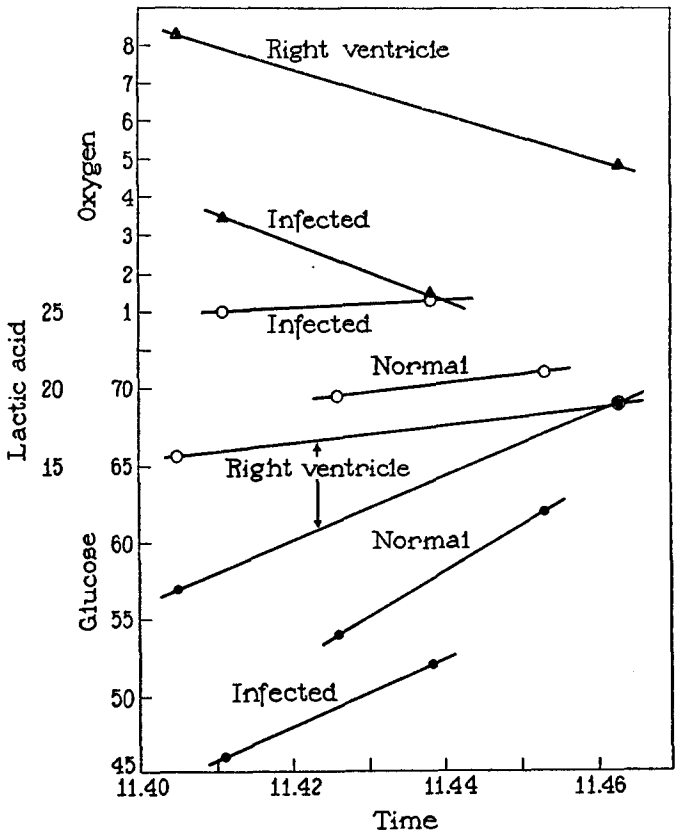

CHART 1. Dog 15. Analysis of blood from the right ventricle and from the normal and infected lobes.

sampling of blood and (*b*) to produce if possible a high degree of oxygen unsaturation of the blood. The latter provided a method of determining whether the metabolism of pneumonic lung tissue could proceed in a normal manner even at low oxygen tensions. Considering the results from both animals, it will be noted first of all that the sugar content of the blood increased in the course of the ex-

periment. This was accompanied by parallel increases of lactic acid.[3] Because of these constant increases it was necessary to collect more than one sample from each site and to note the exact time of collection of each sample.

Dog 15 (see Chart 1) was examined 48 hours after infection. The left lower lobe was completely consolidated and the upper lobes on the same side showed considerable involvement. The entire right lung was normal as was determined by immediate inspection and later by microscopic section. The blood entering the lungs contained only from 8.3 cc. oxygen per 100 cc. at the beginning of the experiment to 4.9 cc. at the end.[4]

TABLE III

Calculations Based upon Interpolated Data from Chart 1 at 11:43

	Right ventricle blood entering lungs	Blood from normal lobe	Blood from consolidated lobe
Glucose, *mg. per 100 cc.*	62	55	50
Lactic acid, *mg. per 100 cc.*	17.0	19.5	25.5
Oxygen, *cc. per 100 cc.*	6.8		2.1
Glucose metabolized, total, *mM per liter*		0.39	0.67
Glucose changed to lactic acid, *mM per liter*		0.08	0.47
Glucose oxidized, *mM per liter*		0.31	0.20
Glucose oxidized, *per cent of total*		80	30
Oxygen consumed, *mM per liter*			2.1
" required by 0.20 mM glucose, *mM per liter*			1.2
" consumed by other metabolites (fats, etc.), *mM per liter*			0.9

Calculations, based upon interpolated data from Chart 1 at 11:43, are shown in Table III. At this time blood from the pulmonary vein of the normal lobe contained 7 mg. per cent less of glucose than the blood entering the lung. The lactic

[3] The hemoglobin content also increased. Thus, in the case of dog 15, the oxygen capacity at 11:40 was 21.8 cc. per 100 cc. and had increased to 31.5 cc. at 11:46. A similar rise was also observed in dog 16, where the oxygen capacity rose from 21.5 cc. at 11:51 to 24.9 cc. at 11:57. This indicates a rapid loss of plasma into the tissues, due perhaps to shock.

[4] It should be remembered that this represents venous blood from the body tissues. The oxygenation in the normal lung was apparently excellent. The oxygen content at the end of the experiment was 29.5 cc. per 100 cc. as compared with an oxygen capacity of 31.5 cc. of blood taken 1 minute later. However the combined blood from the lungs, *i. e.*, mixed blood taken from the left ventricle, at the end of the experiment, contained only 17.7 cc.

acid content was increased only slightly above the experimental error of the method. However, assuming the determined lactic acid value as correct, 80 per cent of the sugar was aerobically metabolized; only 20 per cent appeared anaerobically as lactic acid. This confirms the conclusions reached from the analysis of normal tissue.

The blood from the consolidated lobe still contained a large part of the glucose, while the lactic acid was only moderately increased. The sugar consumption was greater than in the normal tissue, as indicated by a decrease of 12 mg. per cent, and a lactic acid increase of 8.5 mg. per cent. Such an increase was to be expected, because of the large numbers of leucocytes. The metabolism was largely anaero-

TABLE IV

Analysis of Blood 5 Days after Infection, Dog 16

Recovering. Infected lobe ⅓ to ½ consolidated. Blood taken from pulmonary veins.

Blood	Average time of collection	Glucose	Lactic acid	Oxygen	
				Content	Capacity
	hrs.: min.: sec.	*mg. per cent*	*mg. per cent*	*cc. per cent*	*cc. per cent*
Right ventricle	11 : 51 : 11	79.5	16.9	14.6	21.5
Infected lobe	11 : 53 : 36	72.5	19.6	21.1	
Normal "	11 : 56 : 02	72.5	22.9		
Right ventricle	11 : 56 : 50	85.0	25.0	14.7	24.9
Infected lobe	11 : 58 : 34	76.0	28.9	15.4	
Normal "	11 : 59 : 45	85.2	33.2		
Right ventricle	12 : 00 : 35	99.5	35.0	11.4	
Infected lobe*	12 : 06 : 30	92.7	39.2		
Right ventricle	12 : 09 : 47	104.7	44.1		

* Ligature was applied around the pulmonary vein while blood was drawn. It was then immediately released.

bic, due no doubt to the leucocytes. In almost exact agreement with the results from tissues, only 30 per cent of the glucose was oxidized; 70 per cent appeared as lactic acid. Calculated in terms of millimols per liter (see Table III), 0.67 mM of glucose were metabolized, of which 0.47 mM were converted into lactic acid. Glucose oxidized was therefore 0.20 mM. The latter required 0.20 x 6, or 1.2 mM of oxygen for complete oxidation. Our analyses, however, indicate an oxygen consumption of 2.1 mM. This leaves a balance of 0.9 mM for the oxidation of other metabolites.

Dog 16 was studied 5 days after infection. This animal was recovering, as indicated by the x-ray examination, the drop in temperature, and the general improvement. The infected lobe was still one-third to one-half consolidated, but

these areas contained considerable air, as could be judged by the extent of inflation and the later microscopic section. The results fluctuated somewhat, especially those from the infected lobe. Thus at 11:53 the oxygen content of the blood from the infected lobe was 21.1 cc. per 100 cc., indicating almost complete oxygenation, while at 11:58 the oxygen content was slightly higher than the blood entering the lung. Since passage through atelectatic lung tissue, as shown by Adams (17), and through consolidated tissue as shown above by us, results in loss of oxygen, it is apparent that these results indicate access of air. The high degree of oxygenation at 11:53 and the low oxygenation at 11:58 is no doubt due to the method of sampling which in the one took blood from a branch of the pulmonary vein coming from a normal area, while the other represented blood from a not quite recovered area. When plotted, the results indicate a somewhat higher sugar consumption by the normal lung tissue of this dog as compared with dog 15, an average of 11 mg. The blood from the infected lobe contained an average of 13 mg. less of glucose. All of the lactic acid data, within the limits of the method, fall on the same curve. Thus, as was also concluded from the tissue analyses, the recovering tissue consumes only slightly more glucose than the normal tissue, and the results as a whole indicate a return to a completely aerobic metabolism.

DISCUSSION

An important factor in the recovery is the marked change in the type of cells which precedes resolution. This phenomenon has long been known (18). Its relation to the process of resolution and its probable function have been investigated recently by Robertson and coworkers (10, 16). It will be noted from Table I that the relative number of mononuclear cells[5] increased after the 3rd day. Menkin (19), who studied this change in exudates, has suggested that it is due to a rapid depletion of sugar and the development of a high local acidity, all of which may damage the leucocytes; the latter are then replaced by macrophages which are capable of metabolizing under the less favorable conditions. This suggestion is based upon the changes in sugar and lactic acid content and the pH of pleural fluid following injections of turpentine. The exudate, following the initial small dose of turpentine as administered intrapleurally by Menkin, contains mainly leucocytes; no marked accumulation of lactic acid or lowering of the pH is observed. On the 2nd or 3rd day the same or a larger dose of turpentine is administered. This is followed almost immediately by a marked drop in the sugar content, an increase in the lactic

[5] This includes lymphocytes, monocytes, and macrophages.

acid, and a decrease of the pH below 7.0. Macrophages are found to predominate on the succeeding day. The time interval at which the reaction occurs should be noted.[6] Also to be noted in experiments of this kind is the fact that the volume of pleural fluid is large as compared with the absorbing surface; therefore, a rapid interchange between the exudate and the blood is not to be expected. In our experiments, on the other hand, the volume of alveolar exudate is small as compared with the absorbing surface, and the circulation, as we have shown, is adequate to maintain normal metabolism. The number of viable cells, as shown in the last column of Table I, is about the same throughout. Leucocytes are not apparently injured by the conditions which obtain during the first 2 or 3 days, and they disappear at the time when the conditions are returning to normal. Perhaps of greater significance than any possible hydrogen ion changes, is the more prompt macrophage response found in the lungs of animals which have recovered from previous pneumococcus infections (10).

Various factors no doubt contribute to the uneventful and perfect recovery of lung tissue. The pneumonic infection differs in some respects from infections in other tissues; the exudate accumulates in the alveoli, while the tissue itself is relatively little involved. Yet at times many organisms may be seen within the parenchyma, and the latter appears to be decidedly thickened. Toxic substances (20–23, 11) are probably formed by the pneumococcus, but their effect does not seem to be as severe as that of the toxic products of other bacteria. Our results show that the blood supply throughout provides oxygen (even though the latter must be obtained from venous blood) and nutrients, and prevents the accumulation of waste products. The metabolism is thus practically normal throughout, even though the conditions under which the tissue metabolizes have been radically changed. The maintenance of a fairly normal condition throughout is perhaps the most important factor in the complete restoration of the infected tissue.

SUMMARY AND CONCLUSIONS

The metabolism of infected and uninfected lung tissues was determined at various stages of experimental lobar pneumococcus

[6] It is well known that macrophages do not appear in large numbers in exudates induced by such substances as gum arabic until the 2nd or 3rd day.

pneumonia in dogs. Analyses of tissues and analyses of the blood entering and leaving the lungs indicate a fairly normal aerobic metabolism of the tissue throughout the course of the infection.

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